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RESEARCH ARTICLE

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Nickel and copper accumulate at low concentrations in cacao beans cotyledons and do not affect the health of chocolate consumers

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Abstract

Aim of study: Nickel (Ni) and Copper (Cu) are essential metals for the growth and development of plants. In view of the above, the aim of this work was to quantify and correlate Ni and Cu concentrations in the leaf and the parts of the fruit [pod husk, pulp, tegument (seed coating) and cotyledons] of clonal cacao genotype PH 16.

Area of study: Cacao genotypes were collected from adult plants grown on farms located in three different climatic regions of southern Bahia, Brazil.

Material and methods: Plant material was collected in four plots of twenty farms, located under different edaphic and topographic conditions. They were subjected to chemical analysis and later to statistical analyses.

Main results: There was high variability of Ni and Cu concentrations in all evaluated plant materials. Leaf, pulp, and tegument were the plant materials that accumulated more Ni. On the other hand, the greatest accumulation of Cu occurred in the tegument and in the pod husk, while in the cotyledons there was little accumulation of these metals. The concentrations of Ni were influenced by the three climatic regions, a fact not observed for Cu, except at the leaf level. There was interdependence between the accumulation of Ni in the leaves and in the different parts of the fruit, a fact not observed for Cu.

Research highlights: Since Ni and Cu accumulated in low concentrations in the cacao beans cotyledons, raw material for the manufacture of chocolate and other food products, these metallic elements do not affect the consumers' health.

Additional keywords: *Theobroma cacao* L.; fruit; heavy metals; toxicity.

Abbreviations used: H (humid); S (sub-humid); SD (sub-humid to dry).

Authors' contributions: Authors 1, 2, 3, 8 and 9 designed the study, managed the writing of the manuscript and performed the data analysis. Authors 4, 5, 6 and 7 evaluated the parameters analyzed in the study. All authors read and approved the final manuscript.

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Introduction

Cacao (*Theobroma cacao* L.) is grown on 10 million hectares in tropical countries with production of more than four million tons, with beans (fermented seed) being the main commercial product of cacao, which is the raw material for the production of chocolate, cocoa butter, liquor, cosmetics, and medicines (ICCO, 2012). However, only seed cotyledons are used for this purpose, while the tegument (seed coating) results in residue from the chocolate industry, which can be burned for power generation, or used as organic fertilization. Studies on the characterization and evaluation of the cacao bean tegument, aiming at its possible use as a source of nutritional and functional compounds, have been carried out (Vriesmann *et al.*, 2011). From the fruit (pod) of cacao, the pulp (mucilage that surrounds the beans), that can be used for the manufacture of juice, jelly, liquor, wine, and vinegar, can also be extracted. The pod husk, which usually stays in the field, can be used in animal feed and organic fertilization.

Micronutrients Ni and Cu, naturally occurring heavy metals in soils, are essential elements for the growth and development of plants, however, when present in high concentrations can cause harmful effects to plants, environment, and the food chain (Chaves *et al.*, 2010;

Arévalo-Gardini *et al.*, 2016). These metallic elements may be present in cacao beans and in food products in amounts that can affect the health of consumers (Grembecka & Szefer, 2012; Ceko *et al.*, 2014; Bertoldi *et al.*, 2016).

Nickel is an essential element for higher plants due to its important role in the enzyme urease, which is widely distributed in higher plants (Marschner, 2012). This enzyme prevents the accumulation of urea, generated during metabolic processes, which is toxic to plants at high concentrations (Deng *et al.*, 2017). However, when Ni is absorbed in large quantities by plants, it has a toxic effect. Cu is also an essential element for higher plants because it is involved in various physiological processes. This metal participates as a structural element in regulatory proteins, acts on electron photosynthetic transport, mitochondrial respiration, responses to oxidative stress, cell wall metabolism, hormonal signaling, and as an enzymatic cofactor (Yruela, 2009).

The concentration of Cu in plant tissues varies with plant species or ecotypes, developmental stage, and environmental factors such as nitrogen (N) supply and chemical properties of the soil (Yruela, 2009). However, Cu needs to be maintained at low concentrations at cellular level, since this element is extremely toxic, given its high redox properties. Plants grown with high concentration of N requires significantly more Cu, and its bioavailability tends to be higher in acid soils (Yruela, 2009). On the other hand, anthropic actions such as application of copper fungicides to control cacao diseases, such as witch's broom and brown rot, may contribute to the accumulation of this metal in soil and cacao beans (Aikpokpodion *et al.*, 2013; Souza Júnior *et al.*, 2018).

Contamination by heavy metals, besides being one of the greatest threats to ecosystems, presents high toxic potential for human life (Ferrante *et al.*, 2017). Ni is considered carcinogenic, but studies are still incipient about which exposure causes cancer (Clancy & Costa, 2012). The excess of Cu in the body can promote oxidative stress, which also contribute to the emergence of several diseases (Ceko *et al.*, 2014; Scheiber *et al.*, 2014).

In cacao leaves, the concentration of mineral nutrients varies depending on several factors, such as soil fertility and climate (Souza Junior *et al.*, 2018), while the characteristics of the fruits, for the same genetic material, are influenced mainly by the environment (Almeida *et al.*, 2009). Therefore, from the quantification of nutrients in the parts of the fruit (pod husk, cotyledons, tegument, and pulp) and in the leaf, it is possible to evaluate its partition. This allows to obtain information on the nutritional status of the crop and the presence of excesses or deficiencies of elements in its products and by-products.

The objective of this work was to quantify and correlate Ni and Cu concentrations in the leaf and the parts of the fruit [pod husk, pulp, tegument (seed coating) and cotyledons] of clonal cacao genotype PH 16 collected from adult plants grown on farms located in three different climatic regions of southern Bahia, Brazil.

Material and methods

Characteristics of the experimental area

Twenty farms were selected in southern Bahia, Brazil (Fig. 1), located in three climatic regions: humid (H), sub-humid (S) and sub-humid to dry (SD) (Table 1), classified according to typology climatic conditions of the state of Bahia, according to Thornthwaite's methodology (SEI, 2007).

Plant material collection and sample processing

Leaf and fruit collections in plants of the PH 16 clonal cacao genotype, recommended by the Cocoa

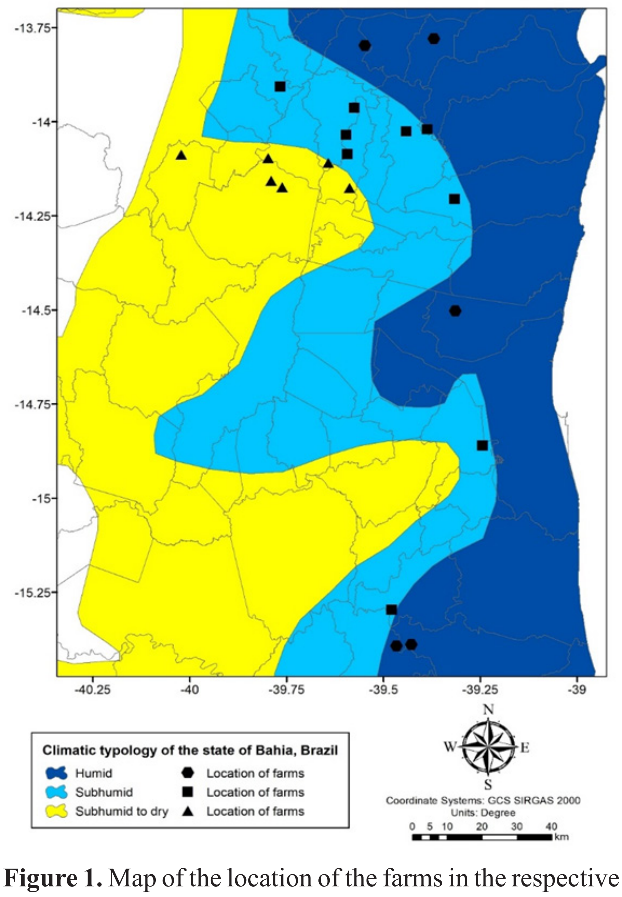

Figure 1. Map of the location of the farms in the respective regions of the South of Bahia, Brazil, according to the climatic typology.

Table 1. Climatic characteristics of cacao producing regions of South of Bahia, Brazil.

Climatic regions	Water index (%)	Water surplus (mm yr^{-1})	Climatic classification[1]
Humid (H)	> 20	600-1200	B4r A’, B3r A’, B2r A, B2r B’, B1rA’ and B1wA’
Sub-humid (S)	0 to 20	50-600	C2bA’, C2bB’ and C2wA’
Sub-humid to dry (HS)	-20 to 0	0-200	C1dA’, C1dB’, C1w2A’ and C1w2B’

[1]SEI (2007).

Research Center (CEPEC/CEPLAC) because its high production and resistance to witches’broom disease (*Moniliophtora perniciosa*) (Leite *et al.*, 2013), aged over six years, were performed in four plots of twenty farms, located under different edaphic and topographic conditions (Fig. 1). Eight mature leaves per plant were collected in four plants per plot from January to February 2012, following the methodology described by Souza Júnior *et al.* (2012), which consisted in collecting the third mature leaf from the apex of newly ripened branches in the four quadrants of the plants. The plant material was packaged separately in paper bags, according to the type of plant material, farm and region, and taken to the laboratory. The leaves were then washed with distilled water and then placed in an oven with forced air circulation at 65 °C until reaching constant mass. Finally, the samples were milled with a Willey type MA-340 mill, using 20 mesh sieves.

Four mature fruits per plant were collected, which were broken apart, separating them in husk and beans (seeds). The placenta remained attached to the husk. Seed pulp was removed with the aid of a plastic sieve, and the pulps were packed in plastic containers and frozen for further analysis. Beans and pod husk were dried in an oven at 60 °C, with forced air circulation, until reaching constant mass. Subsequently, the dried beans were separated into cotyledons and tegument, with the aid of a scalpel.

Chemical analysis

The chemical analyzes were adapted from the methodology described by EMBRAPA (2000) which uses samples of 0.20 g of natural pulp and dry matter of leaf, husk, tegument and cotyledons were digested with 4 mL of nitric acid and 3 mL of hydrogen peroxide. The digestion was performed in a digester block with initial temperature of 50 °C for 30 min and final temperature of 120 °C for 90 min. Afterwards, the samples were added into falcon tubes to 14 mL and Ni and Cu readings were measured by inductively coupled plasma atomic emission spectrometry model Varian 710-ES (Mulgrave, Austrália), and the analyses were performed in duplicate. This equipment is equipped with a radio frequency generator of 40 MHz used in the 1200-1400 W range for the proposed study and a solid state with charge coupled device (CCD) (Moreira, 2016).

Statistical analyzes

Data, by climatic region, were subjected to descriptive statistics analysis and Shapiro Wilk's normality test (W) (p<0.05), and the means were compared by the Tukey test (p<0.05). The concentrations of Ni and Cu in the leaves and in the different parts of the fruit were submitted to Pearson's linear correlation.

Results and discussion

Nickel

In general, for all three climatic regions, the mean concentrations of Ni in the leaves and in all parts of the fruit were close to the medians (Table 2), indicating that the average would be a good reference of the central value of the sample. Larger differences between the mean and median values were observed only in the S region for leaf and cotyledons.

For all the evaluated plant materials, the concentrations of Ni presented normal distribution in the H region, according to Shapiro Wilk's test, a fact opposite to that observed in the S region (Table 2). In the SD region, only for leaf and pulp, the concentrations of Ni presented normal distribution (Table 2). According to Cressie (1991), data normality is not a requirement for a good set of data as long as the normal distribution curve does not have very elongated lines (high amplitude). This fact was not evidenced in the present work, since the similarities of the values of central tendency (average and median) indicate symmetrical distributions.

According to the classification proposed by Warrick & Nilsen (1980), the coefficient of variation (CV) was high for all variables, except the pod husk (region SD), cotyledons (region H), and tegument (region S), which presented average CVs (Table 2). These results indicate a wide variability in Ni concentrations in all analyzed plant materials. This fact was corroborated by the high amplitudes (differences between the minimum and maximum values) of the concentrations of Ni in the leaf and in the parts of the fruit (Table 2). Probably the high

Table 2. Analysis of the descriptive statistics and test of mean of Ni concentrations in the leaves and different parts of the fruit of PH 16 clonal cacao genotype as a function of the regions H, S and SD of the state of Bahia, Brazil.

Region	Minimum	Maximum	Mean	Median	CV (%)	W
	----	----	($mg\ kg^{-1}$)	----		
			Leaf			
H	11.0	247.0	105.5^{b}	102.5	70.6	ns
S	8.0	216.0	59.8^{b}	41.5	85.4	*
SD	9.0	666.0	226.7^{a}	218.5	75.1	ns
			Husk			
H	0.8	18.1	7.2^{a}	6.3	64.9	ns
S	0.3	30.8	7.4^{a}	6.6	78.3	*
SD	3.6	24.8	10.0^{a}	8.6	57.2	*
			Cotyledons			
H	0.5	6.7	3.5^{b}	3.9	51.3	ns
S	0.6	15.0	4.2^{b}	2.9	89.2	*
SD	1.1	19.9	8.0^{a}	5.4	68.9	*
			Tegument			
H	12.4	200.0	85.0^{ab}	85.4	63.9	ns
S	14.3	112.8	50.1^{b}	42.6	56.7	*
SD	11.4	320.5	103.4^{a}	93.8	66.9	*
			Pulp			
H	3.0	72.0	34.9^{b}	41.5	62.5	ns
S	3.0	186.0	25.2^{b}	18.5	75.1	*
SD	4.0	304.0	109.5^{a}	101.5	75.2	ns

ns and *: Normal and non-normal distribution by Shapiro Wilk's test ($p<0.05$), respectively. For a given plant material, different letters in the column indicate statistical difference by the Tukey test ($p<0.05$).

CV values and high amplitudes of Ni concentrations in the plant are due to the high geological and pedological variability of the cacao producing region of the South of Bahia and, consequently, high variability of the mineralogical, chemical, and physical attributes of the soils (Arévalo-Hernández *et al.*, 2019). The variation of Ni concentration in the soil strongly depends on the source material (Massoura *et al.*, 2006). According to these authors, when analyzing the concentration of Ni in soils of various parts of the world, there is a wide variation of the concentrations of this metal in the soil, which varied from 4 to 2000 mg Ni kg^{-1} soil, being the highest concentrations found in soils derived of ultramafic rocks.

There was significant difference of the mean concentration of Ni between the climatic regions, for the leaf and in all evaluated parts of the fruit, except for husk. The mean concentrations of Ni in leaf, cotyledons, and pulp were higher in the region SD in relation to the other regions. However, for tegument, the mean concentration of Ni in the region SD was significantly higher only in relation to the mean of the S region (Table 2). The highest values of Ni in the analyzed plant materials found in the region SD may be due to the fact that this region has less aged soils than the other two climatic regions; being these more coastal (Fig. 1). In the region SD, there is also presence of ultramafic rocks, with mineral exploitation of Ni, with the mine being considered the third largest open pit of sulfided nickel in the world (Matta, 2016).

Arévalo-Gardini *et al.* (2017), analyzing heavy metals in the eight major cacao growing regions in Peru, did not find significant differences between regions for Ni concentrations in leaves and in cacao beans. According to these authors, mean concentrations ranged from 2.2 to 12.2 mg Ni kg^{-1} DM and from 3.5 to 9.2 mg Ni kg^{-1} DM, for leaf and beans, respectively. In turn, Bertoldi *et al.* (2016) studied the geographical chemical traceability, based on the analysis of 56 elements of cacao bean samples from the 23 main cacao producing countries of different continents (Africa, Asia, Central and South America). These authors observed that the average concentration of Ni in the beans, in the Central American samples, was 12.1 mg Ni kg^{-1} DM, which is significantly higher than the average values found in the other continents, which presented averages ranging from 4.9 to 6.7 mg Ni kg^{-1} DM.

Bertoldi *et al.* (2016) and Arévalo-Gardini *et al.* (2017) described in their methodological procedures that the beans were peeled and separated in tegument and cotyledons, and the Ni concentration in the tegument was approximately 15 times higher than the cotyledons (Table 2). This shows that the Ni present in cacao beans accumulates predominantly in the tegument, which is not used in the food industry, being an industrial residue. Only the cotyledons are used for the production of liquor, chocolate, cocoa butter and its derivatives. On the other hand, the pulp that surrounds cacao beans, which can also be used in the food industry, had higher Ni concentrations. Although, the pulp was the only part of the fruit analyzed based on the natural matter, while the other vegetal materials were analyzed based on the dry matter.

In the 80 foliar samples analyzed in the present study, the concentration of Ni varied greatly from 8 to 666 mg Ni kg^{-1} DM (Table 2), with average values being much higher than those observed by Arévalo-Gardini *et al.* (2017). Phytotoxic Ni concentrations vary widely among plant species and cultivars, and there are reports of phytotoxic foliar concentrations ranging from 40 to 246 mg Ni kg^{-1} DM. However, there are native plants that grow in soils naturally contaminated with Ni, which have concentrations in the leaves above 6000 mg Ni kg^{-1} DM (Kabata-Pendias, 2011). However, for cotyledons, the amplitude of Ni concentrations was much lower than in the leaf, ranging from 0.5 to 19.9 mg Ni kg^{-1} DM (Table 2). These values are similar to the variations found in cacao beans by Arévalo-Gardini *et al.* (2017) and ratified by the concentrations of 5 to 10 mg Ni kg^{-1} DM, presented by Kabata-Pendias (2011), as commonly found in a cocoa powder sample.

In order to compare the partition of the two metals in the analyzed plant materials based on dry matter, the leaf was adopted as the reference organ, since it is the organ-spot of plant metabolism and often used to evaluate the nutritional status of the plant (Souza Júnior *et al.*, 2018). Considering the average Ni concentration of the three studied regions, in each plant material, the concentrations of Ni in the tegument, pod husk and cotyledons were, respectively, 60.9%, 6.3% and 4.0 % of the average concentration found in the leaf (Table 2), evidencing the dilution effect of this metal on the fruit. This indicates, in turn, the low mobility of this metal from the leaf to the fruit, and in the fruit, it was more concentrated in the tegument than in the pulp (Table 2).

Nickel is a relatively mobile element in the phloem and can be readily transferred from sources (mature leaves) to preferential metabolic drains such as leaves and young fruits (Page & Feller, 2005; Page *et al.*, 2006). When it reaches the draining organs, Ni can be exported from the tissues of the phloem to the apoplast again, where it is absorbed by neighboring cells (Deng *et al.*, 2017). The translocation of Ni via phloem is bidirectional, including downward and upward motions. Deng *et al.* (2016) verified that 89% of Ni are exported from mature leaves to young leaves of *Noccaea caerulescens*, while only 11% are exported from mature leaves to roots. This suggests that upward movement is the predominant direction for translocation in the phloem, and that young leaves and reproductive organs are the primary preferential metabolic drains of Ni via phloem (Deng *et al.*, 2017). Moreover, Estrade *et al.* (2015) also observed that the fractionation of Ni isotopes between leaves and flowers occurs in the initial stages of growth of *Alyssum murale*, resulting from the net transfer of Ni to the leaves. According to these authors, in the stage of full bloom, the Ni isotopic compositions between leaves and flowers are leveled, indicating that the great redistribution of phloem occurs at this stage.

Studies with radioactive ^{63}Ni, applied via foliar in different plant species, demonstrated that Ni can be rapidly transferred to young leaves and young seeds (Fismes *et al.*, 2005; Riesen & Feller, 2005). This fact was also observed in Ni hyperaccumulating plants, which accumulate this metal in leaves, flowers and seeds at high concentrations (Zhang *et al.*, 2014; Groeber *et al.*, 2015). As the reproductive organs are the main translocation drains of the phloem, this evidence indicates that hyperaccumulating plants can carry substantial amounts of Ni via phloem. The high concentration of Ni in the phloem sap was documented in several Ni hyperaccumulating plant species growing in tropical ultramafic soils, such as *Euphorbia helenae* (3.1%) (Reeves *et al.*, 1996) and *Phyllanthus balgooyi* (16.9%) (van der Ent & Mulligan, 2015; Mesjasz-Przybylowicz *et al.*, 2016). However, in the present work, young leaves of cacao are probably the preferential metabolic drains for Ni from mature leaves, to the detriment of young fruits, due to the low mobilization of Ni from mature leaves to cacao fruits. Although there are no new branches during the fruiting of cacao trees, pruning, used to eliminate dry brooms, caused by the fungus *Moniliophthora perniciosa*, interferes with the phenology of the plant, breaking the apical dominance of the branches and stimulating the appearance of new leaves concomitant with the fruiting.

Positive and significant correlations of Ni concentrations among all the studied plant materials were observed (Table 3). This demonstrates interdependence between the accumulation of Ni in the leaves and in the different parts of the fruit. It also suggests that the leaf, which is already analyzed for plant nutritional

Table 3. Correlation of Ni concentrations in different parts of fruit and leaf of clonal cocoa genotype PH 16.

	Husk	Cotyledons	Tegument	Pulp
Leaf	0.31**	0.59**	0.71**	0.78**
Husk		0.28**	0.28**	0.24*
Cotyledons			0.49**	0.70**
Tegument				0.75**

** Significant correlation at 5% probability by t-test.

diagnosis, can also be used as an indicator of the accumulation of Ni in parts of the fruit used for food production, such as cotyledons and pulp.

The upward movement of Ni to the aerial part follows the flow of the xylem, which final destinations are mature leaves, due to the high transpiration in these tissues, while young leaves receive a small proportion of Ni via xylem (Deng *et al.*, 2017). The Ni transported via xylem is in the form of free hydrated cations, and when it reaches the smaller veins and fills the apoplastic spaces in the leaves, it is loaded in the foliar symplast or remains in the apoplast (Deng *et al.*, 2017). In leaves, Ní is preferentially distributed in epidermal cells, at least in the active tissues of the symplast (Tappero *et al.*, 2007). In leaf symplast, Ni is rapidly transferred to vacuoles, particularly in epidermal cells, where it is chelated mainly by carboxylic acids (Deng *et al.*, 2017). The mesophilic paralisade cells become an important compartment when there is increase in Ni concentration in the leaves (Broadhurst *et al.*, 2004).

Copper

For all the analyzed plant materials, the values of central tendency measurements of Cu concentrations presented similar behavior to those of Ni, that is, similar mean and median values. Almost all Cu data presented a non-normal distribution, except for pod husk, in the three regions, and for cotyledons and leaf in the region H (Table 4). Regardless of the analyzed plant material, CV for Cu were lower than those observed for Ni (Tables 3 and 4), indicating a lower variability of that. According to the classification presented by Warrick &

Table 4. Analysis of descriptive statistics and test of means of Cu concentrations in different parts of fruit and leaves of clonal cocoa genotype PH 16 as a function of the regions H, S, and SD in the state of Bahia, Brazil.

Region	Minimum	Maximum	Mean	Median	CV %	W
	----------------------	(mg kg^{-1})	-------------------			
			Leaf			
H	7.9	21.2	12.8^a	11.8	31.2	ns
S	3.5	30.6	9.9^b	9.4	45.1	*
SD	5.2	31.7	8.9^b	7.1	64.4	*
			Husk			
H	93.0	227.5	149.0^a	152.4	25.7	ns
S	95.2	208.9	155.3^a	160.0	16.9	ns
SD	75.2	229.4	147.7^a	131.3	26.9	ns
			Cotyledons			
H	6.1	19.1	11.1^a	11.2	27.3	ns
S	2.5	29.7	12.2^a	9.6	58.1	*
SD	4.2	32.3	13.4^a	8.5	62.6	*
			Tegument			
H	119.9	457.3	227.3^a	209.1	34.7	*
S	65.8	578.5	171.2^a	165.2	48.0	*
SD	79.5	350.4	165.3^a	161.3	34.9	*
			Pulp			
H	6.8	25.4	11.7^a	11.2	40.1	*
S	4.3	29.9	13.1^a	12.3	42.3	*
SD	5.8	45.5	15.5^a	14.2	55.2	*

ns and *: Normal and non-normal distribution by Shapiro Wilk's test ($p<0.05$), respectively. For a given plant material, different letters in the column indicate statistical difference by the Tukey test ($p<0.05$).

Nilsen (1980), the great majority of CV for Cu fits as average.

There was not significant difference in Cu concentrations between the climatic regions in any of the fruit parts. However, the mean concentration of Cu in the leaves of the region H was significantly higher than the means of the other two regions (Table 4). This fact can be attributed to the greater use of copper fungicides in that region for the control of brown rot and witch broom diseases (Veloso & Santana, 2000). However, there are reports of Cu deficiency in cacao in the region SD (Souza Júnior *et al.*, 1999). Considering the appropriate leaf range of 10 to 20 mg Cu kg^{-1} DM, for cacao trees grown in the South of Bahia (Souza Júnior *et al.*, 2018), of the 80 samples analyzed in the three regions, 60% of the plants would be deficient in Cu, 48.3% with adequate concentrations and only 3.7% with excessive concentrations. In addition, the highest frequency of deficiency occurred in the regions SD and S, which had 83.3% and 58.3% of plants with Cu deficiency.

On average, for the three regions together, Cu concentrations in the pod husk, tegument and, cotyledons were 1334%, 1685%, and 16%, respectively, higher than the Cu leaf concentration (Table 4). It evidences the effect of concentration of this metal on the fruit, which indicates that Cu would have good mobility in phloem for cacao trees. However, it would focus on fruit residues (husk and tegument) and not on parts frequently used in the food industry, such as cotyledons and pulp (Table 4). Arévalo-Gardini *et al.* (2017) also observed, in eight cacao producing regions in Peru, Cu concentrations in cacao leaves lower than those found in cacao beans (tegument + cotyledons), ranging from 7.2 to 10.3 mg Cu kg^{-1} DM and 18.8 to 30.4 mg Cu kg^{-1} DM, respectively. On the other hand, different from that observed for Ni (Table 3), no significant correlations were observed for Cu concentrations among the different evaluated plant materials.

In plants, Cu can be transported in the form of free Cu (I) or Cu (II), however, it is usually transported as Cu complexes (Yruela, 2009). There is strong evidence that Cu, once in the xylem sap, is transported in Cu (II) form by specific metal chelators (Printz *et al.*, 2016). Possible ligands candidates are small molecules, including carboxylates of organic acid such as citrate and malate, amino acids [nicotianamine (NA), histidine (His) and cysteine (Cys)], high affinity Fe (III) chelating compounds, and NA derivatives called phytosiderophores, such as muginoic and 2-deoximuginic acids, as well as peptides and proteins [metallothioneins (MTs)] (Alvarez-Fernandez *et al.*, 2014). At pH of the xylem sap, NA and His are the major Cu ligands, suggesting that these amino acids are the major ligands during the long-distance transport of Cu in the xylem (Pich & Scholz, 1996).

MTs are Cys-rich proteins capable of coordinating the Cu (I), Zn (II) and Cd (II) ions by their thiol groups (Wan *et al.*, 2013). These proteins are involved in the redistribution of Cu from senescent leaves to draining organs such as young leaves or seeds in development (Benatti *et al.*, 2014). NA is ubiquitous in higher plants and present in all tissues, and is involved in metal transport (Clemens *et al.*, 2013). Histidine has three metal binding sites (carboxylate, α-amino, and imidazole groups). The last group forms strong bonds and strong complex, especially with Ni and Cu (Alvarez-Fernandez *et al.*, 2014). There is evidence that His is involved in the long-distance transport of metals in plants, mainly Ni in the xylem of hyperaccumulating species of the genus *Alyssum* (Deng *et al.*, 2017). His (in the µM-mM range) and Ni concentrations in the xylem sap are significantly and linearly correlated with various Ni hyperaccumulating *Alyssum*, such as *A. lesbiacum*, in response to the increase of concentrations of metals in the growth media (Krämer *et al.*, 1996).

In general, leaf, tegument, and fruit pulp of clonal cacao genotype PH 16 were the plant materials that accumulated more Ni. The largest accumulation of Cu was in the tegument (cacao beans/seeds coating) and in the pod husk, whereas in the cotyledons there was little accumulation of these metals. There was high variability of Ni and Cu concentrations in all the analyzed plant materials. The concentrations of Ni were influenced by the climatic region, a fact not observed for the Cu, except for its foliar concentration. There was interdependence between Ni accumulation in the leaves and in the different parts of the fruit, a fact not observed for Cu. The low Ni and Cu accumulation in cacao beans cotyledons, raw material for the manufacture of chocolate and other food products, has shown that these metallic elements do not affect consumers' health.

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